Clinical study

Adaptive Randomized trial for therapy of COrona virus disease 2019 at home with oral antivirals (ARCO-Home study)

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1. Synopsis

1.1. Background

At the end of December 2019, a new pandemic SARS-like-CoV (SARS-CoV-2) has emerged in China and rapidly spread across the globe. Italy is currently the country with the largest local epidemic outside China. Figures suggest that cumulative incidence exceeds 2 case per 1000 inhabitants in several areas of the country. Moreover, the lethality rate in Italy is the highest ever registered in countries with active surveillance system for COVID-19. The frail structure of the Italian population, combined with the astonishing intensity of the epidemic wave, is the main reasons for this overwhelming circumstance. Besides, the unprecedented over-working faced by hospitals at local level could have contributed to amplify the appalling effect of the epidemic. In this situation, new strategies that can mitigate the impact of COVID-19 on the National Health System are urgently needed. Specifically, these strategies should be aimed at: A) preventing patients with mild COVID-19 to progress towards severe/critical disease, for lightening the impact on hospitals; B) shortening time of viral shedding in order to curb individual infectivity and reduce the average number of secondary cases. At present, there are neither standard approaches nor approved drugs to care patients with COVID-19 and intensive care life-support therapies represent the only effective intervention to reduce mortality for patients with critical COVID-19. Several antivirals have been currently proposed as potential effective therapies against COVID-19. Among the most promising compounds there are anti-HIV PIs, including Lopinavir-ritonavir (LPV-r) and Darunavir-cobicistat (DRV-c), a broad-spectrum inhibitor of viral RNA polymerase, Favipiravir (FAV) and Hydroxychloroquine (HCQ).

1.2. Objectives

The aim of this study is to assess the efficacy of four anti-virals (LPV-r, DRV-c, FAV and HCQ) for preventing the evolution of COVID-19 towards severe and critical disease and their capability to reduce the mean time of viral shedding in COVID-19 patients who do not need immediate hospital admission.

1.3. Methods

The study is designed as a repurposing phase 3 adaptive trial implemented during a massive epidemic emergency. The adaptive strategy will consist of a parallel multi-arm open random comparison against a standard of care. This includes a 4-stage sequential design; sample size re-calculation at each stage and stopping rule for efficacy with a pick the winner strategy. The study will be conducted in 5 Italian Provinces (Rome, Verona, Parma, Piacenza and Cremona), but, in principle, it can be immediately extended to other setting within and outside Italy. Study population will include male and non-pregnant female adults aged 18 years or older with COVID-19.

1.4. Expected results

The ongoing SARS-CoV-2 epidemic makes it clear that our current capability to manage clinical cases of COVID-19 is very limited. This multi-arm multistage adaptive trial is expected to provide a crucial answer about the actual efficacy of four antivirals on the treatment of mild and moderate COVID-19. The trial is expected to enroll on average 175 and 435 participants and be concluded in about three months. The trial will have a direct impact on future use of PIs in the therapy of COVID-19. Besides, if the trial will prove the superiority of PIs on standard of therapy the impact on healthcare system will be significant. In fact, preventing severe COVID-19, by using a safe all-oral treatment will be a terrific toll for mitigating the effect of the epidemic at national and international level.

2. Background

Severe-acute-respiratory-syndrome-like-coronaviruses (SARS-like-CoV) have caused major outbreaks of fatal pneumonia since the beginning of the 21st century. Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) spread to five continents in 2003 with a fatality rate of 10%. [1] Eventually, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) broke out in the Arabian Peninsula in 2012 with a fatality rate of 35%. [2] At the end of December 2019, a new pandemic SARS-like-CoV has emerged in China and rapidly spread across the globe.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is associated with a new diverse clinical syndrome called Coronavirus disease 2019 (COVID-19). Common symptoms of COVID-19 include fever, cough, shortness of breath and dyspnea that may eventually progress towards acute respiratory distress syndrome and death. About 80% of patients have mild to moderate disease, 14% have severe disease and 6% are critical (namely, they develop respiratory failure, septic shock, and/or multiple organ dysfunction/failure). [3-4] The severity of clinical presentation is directly dependent on patients' age. Infections needing hospitalization are exceptional events among children younger than 10 years while the case-fatality rate can exceed the 10% among people older than 70 years. [5-6]

Italy is suffering the worst epidemic event in its modern history. Current figures suggest that several areas in the northeast of the country have been experiencing a massive intensity epidemic with a cumulative incidence exceeding 5 cases per 1000 inhabitants. Despite Italy has a well-structured healthcare system, the impact of COVID-19 has been heavy. The World Health Organization (WHO) reported figures suggesting that lethality rate in Italy is the highest ever registered in countries with active surveillance system for COVID-19. The reasons for this overwhelming circumstance are diverse and may encompass the frail age structure of the Italian population, the fiery pace of the epidemic and hospital overworking. In particular, recent estimates suggest that Italy has approximately 5200 beds in intensive care units. Given that the mean time of COVID-19 in intensive care unit is about 2-3 weeks, we should be prepared for a major stress on critical care facilities in our hospitals. [7]

There are three pillars for mitigating the impact of this epidemic. First, implement (social) non-pharmacological interventions to curb transmission. Second, reorganize hospitals in order to absorb the blast when the epidemic peaks. Finally, produce innovative pharmacological strategies for either preventing new infections or at least adverting most severe clinical presentations in COVID-19 cases.

Proteases inhibitors (PIs) are a class of all-oral antiviral drugs with a high profile of safety. PIs affect viral production by interfering with viral proteins maturation. Many viruses, including SARS-CoV-2, produce mature functional proteins by hydrolyzing large polyprotein precursors. Recently, molecular docking analyses have suggested that several PIs, currently used in anti-HIV therapy, may bind and inactivate SARS-CoV-2 major proteases (3C-like-protein; 3CLpro). [8] Favipiravir (FAV) [9] is a broad-spectrum RNA-dependent-RNA polymerases (RdRp) inhibitors active against different genera of RNA viruses. Its activity against SARS-CoV-2 has been suggested by *in virtro* activity and preliminary results of clinical studies carried out in China. [10-11]

Chloroquine, largely used as treatment for malaria, is known to block virus infection by increasing endosomal pH required for virus/ cell fusion, as well as interfering with the glycosylation of cellular receptors of SARS-CoV [12]. A recent study shows that chloroquine is highly effective in the control of 2019-nCoV infection in vitro, demonstrating that it functioned at both entry, and at post entry stages of the 2019-nCoV infection in Vero E6 cells. Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo (13).

This protocol contains a proposal to develop an adaptive repurposing trial for assessing the efficacy of four antivirals (including 2 anti-HIV PIs, FAV and HCQ) to reduce infectivity and clinical progression of subjects with mild and moderate COVID-19, who do not need immediate admission in hospital at diagnosis.

3. Rationale

Molecular docking is a method for predicting the level of chemical affinity and virtual strength of binding between two molecules and thus may serve to obtain fast and convenient surrogates for *in vitro* drug testing of efficacy. These analyses suggest that several PIs [14-15] currently used for antiretroviral therapy, including lopinavir (LPV) and darunavir (DRV) can bind and inactivate 3CLpro, a molecular target essential for the replication of the β-CoVs.[16] These PIs are oral drugs conveniently co-formulated with a booster for improving pharmacokinetic profiles. Clinical experience with HIV demonstrated that boosted PIs, including LPV-ritonavir (LPV-r) and DRV-cobicistat (DRV-c) have high safety profile and optimal pharmacokinetic performance.

FAV is a broad-spectrum RdRp inhibitor. *In vitro* studies suggest that FAV is capable of blocking the replication of flavi-, alpha-, filo-, bunya-, arena-, noro-, and other RNA viruses. FAV is converted into an active phosphoribosylated form (FAV-RTP) in cells and is recognized as a substrate by viral RdRp, thus inhibiting RNA polymerase activity. Therefore, FAV may have potential antiviral action on SARS-CoV-2, which is an RNA virus. [9]

CoVs are enveloped viruses with a positive RNA genome, belonging to the Coronaviridae family including four genera (α, β, γ, and δ). Similarly, to other SARS- like-CoVs, SARS-CoV-2 belongs to the β genus. β-CoVs genome is one of the largest among RNA viruses being between 29 and 34 Kb. β-CoVs express 5 structural and up to 16 non-structural proteins. Knowledge on SARS-CoV-2 are still incomplete but most recent evidence suggests that it is very similar to other β-CoVs including SARS-CoV-1 and MERS. [17] β-CoVs produce mature functional proteins by cleaving them from its two overlapping “polyproteins,” pp1a (486 kDa) and pp1ab (790 kDa). Papain-like protease 2 (PL2pro) and 3CLpro are both essential for viral replication. PL2pro cleaves three sites and 3CLpro cleaves 11 sites. Animal studies [18] suggested that without the combined action of these enzymes β-CoVs cannot produce individual functional proteins that are essential for viral replication, including an RdRp, helicase and the single-stranded RNA-binding protein. [19] Several evidences suggest that PIs, FAV and HCQ can be active against-CoV diseases. LPV is active against SARS-CoV-1 and MERS. [20] Moreover, CoVs strains with 3CLpro mutants resistant to PIs are attenuated for replication and pathogenesis suggesting that, similarly to HIV, resistance to PIs can occur at the cost of viral fitness. [21] Animal models suggest that inhibition of 3CLpro pathway trough PIs is strongly associated with recovery also in critically ill animals. [22-23] Finally, in vitro study suggest that FAV may inhibit SARS-CoV-2 RdRp and thus prevent viral replication. [9] Chloroquine seems to act both as antiviral and as immunomodulant. It blocks virus infection by increasing endosomal pH required for virus for the fusion with the cell and it interferes with the glycosylation of cellular receptors of SARS-CoV [24]. A recent study shows that chloroquine is highly effective in the control of 2019-nCoV infection in vitro, demonstrating that it functions at both entry, and at post entry stages of the 2019-nCoV infection in Vero E6 cells. Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo [25]. Chloroquine is widely distributed in the whole body, including lung, after oral administration. The EC90 value of chloroquine against the 2019-nCoV in Vero E6 cells was 6.90 μM, which can be clinically achievable as demonstrated in the plasma of rheumatoid arthritis patients who received 500 mg administration [26].

Clinical evidence on efficacy of PIs FAV and HCQ on COVID-19 are sparse and inclusive. Most recent published evidence from Singapore (18 patients) [27] and China (10 patients) [28] suggested that administration of LPV-r is associated with improvement of clinical condition. However, a recent controlled trial enrolling 199 participants found that addition of LPV-r to standard of care produces no benefit in hospitalized adult patients with severe COVID-19 who started treatment later than a week since symptoms onset. [29] However, potential efficacy of LPV-r in early treatment of COVID-19 remain unexplored.

There is no direct evidence that FAV is better than standard of care (without antivirals) in therapy of COVID-19. A nonrandomized study carried out in China suggests that co-administration of IFN-alpha plus FAV is associated with better clinical outcome than co-administration IFN-alpha plus LPV-r in patients with severe COVID-19.[10] Moreover, a recent clinical randomized study provide evidence that patients receiving FAV had a shorter viral shedding than those receiving Umifenovir (arbidol); however the study provide no evince for better clinical outcome of FAV in comparison to Umifenovir.[11]

There is a consensus that therapy with HCQ should be considered for patients with COVID-19 on the ground of in vitro data. [30] However, at present the only peer-review evidence of the efficacy of HCQ against COVID-19 *in vivo* come from a small nonrandomized study in France. [31]

There are no published data for either DRV-c. Nevertheless, molecular docking analyses predict that DRV-c can be more active against SARS-CoV-2 than LPV-r. On the light of high tolerability and potential activity, PIs represent optimal candidates to reduce infectivity and clinical progression of subjects at the early stage of COVID-19. It is noteworthy that DRV-c trials have been already started (Shanghai Public Health Clinical Center NCT04252274, Rajavithi Hospital Thailand NCT04303299; Public health Catalunya NCT04304053. [32]

4. Impact for the National Health System

Italy has been the first European country to be heavily stricken by the COVID-19 epidemic. Despite Italy has a well-structured healthcare system, the impact of COVID-19 is heavy due to the fiery pace of the epidemic progression in several crucial areas and for the frail age structure of the Italian population. In fact, topical evidence suggests that older age and co-morbidities are the strongest predictor of progression toward severe and critical COVID-19 needing either hospital or intensive care, respectively.

According to the EU commission, Italy is the country with the highest median age among the 28 EU Member States in 2018. The EU reported that median age in Italy is 46.3 years in comparison with an EU-28 average of 43.1 years. Furthermore, a detailed analysis of the Italian population structure indicates that 22.6% people are older than 65 years and that 7% are older than 80. [33] This observation is also consistent with the high-observed prevalence of several clinical co-morbidities associated with aging. The Italian Society for hypertension reports that more than one third of the Italians suffer from hypertension. [34] Similarly, the Italian National Institute of Statistics estimates that about 5% of Italian people are diabetic; this percentage rises to 16.5% for those who are older than 65 years. [35]

The peculiar structure of the Italian population combined with of the astonishing intensity of the epidemic wave in several areas have already resulted in a significant lethality, which is currently much higher than that ever reported in any other country with an active surveillance for COVID-19. Experts suggest that the unprecedented over-working faced by hospitals at local level could have contributed to amplify the appalling effect of the epidemic. [7] On March 9, to prevent the

occurrence of an unmanageable epidemic wave, the Italian government has enacted a special legislation. This legislation endorses social distancing, closure of all non-essential social and economic activities, reinforcing central system of surveillance and support for local health structures. Several other European countries (e.g. France, Spain and Belgium) have eventually followed this policy. Even though these actions seem to represent the only effective measures to contain further COVID-19 spreading, the social and economic impact on people life quality and global economy will be very high.

In a setting made of many frail people with the pivotal need of restarting economic activities while maintaining a high level of safety for all people, there is the urgent need to find new therapies that can actually mitigate the impact of COVID-19 on the National Health System. The most urgent needs are:

A. Preventing patients with mild COVID-19 to progress towards severe/critical diseases, for lightening the impact on hospitals;
B. Shortening time of viral shedding in order to curb individual infectivity and reduce the average number of secondary cases.

Our trial is specifically meant to this. We will test the efficacy of four antivirals for preventing the evolution of COVID-19 towards severe and critical diseases and their capability to reduce the mean time of viral shedding. These parameters are strongly associated with the potential impact of COVID-19 on the National Health System. In fact, individual infectivity is directly associated with disease severity and time of viral shedding. Moreover, preventing severe COVID-19 will directly reduce lethality and will immediately mitigate the hospitals overworking. Besides, the trial is will serve to re-enforce home care, to redefine a standard of care for mild-moderate COVID-19 cases and to advice general practitioners on the potential efficacy of safe and all-oral drugs, such as PIs, against COVID-19.

5. Objectives of the study

The ongoing SARS-CoV-2 epidemic makes us painfully realize that our current capability to manage clinical cases of COVID-19 is very limited. Even if the previous outbreaks of SARS-CoV-1 in 2003 and MERS-CoV in 2012 triggered extensive research efforts, there are currently neither standard approaches nor approved drugs to care patients with COVID-19. In fact, due to the transient nature of CoVs epidemics, no prototype anti-CoV drug has progressed to the clinical stage to date. At present, intensive care life-support therapies represent the only effective intervention to reduce mortality for patients with critical COIVID-19.

The objective of this study is to assess whether LPV-r, DRV-c FAV or HCQ are superior to a standard of care in reducing disease duration and disease severity in patients that are diagnosed with COVID-19 and do not need immediate hospital admission.

In a series of 18 consecutive cases of COVID-19, from Singapore, the median duration of viral shedding was 12 days (range 1-24) and 15 patients (83%) had viral shedding for 7 days or longer. [27] In a pivotal cohort study with 191 subjects carried out in two Chinese hospitals the median duration of viral shedding in survivors was 20.0 days (IQR 17.0–24.0) while in non-survivors the virus was continuously detectable until death. [36] Among the 29 survivors who received LVP-r the median time of viral shedding was not significantly different from those who did not receive LPV-r but the median time of treatment initiation was about 14 days (IQR 10.0–17.0).

A recent analysis carried out on Lazio Regional System (Coronet) suggests that the rate of hospital admission among COVID-19 cases confirmed since 2 April 2020 was 44% and the median time to hospitalization since fever onset was about 7 days.

5.1. Primary Objectives

The primary objectives of the study is to assess whether early therapy with LPV-r, DRV-c FAV or HCQ can either reduce the time of viral shedding or the need of hospitalization in mild and moderate COVID-19.

Early therapy is a therapy with LPV-r, DRV-c FAV or HCQ that start within 5 days since symptoms onset. *Mild and moderate* cases of COVID-19 are those patients that do not need an immediate hospital admission at time of the diagnosis.

5.2. Other objectives

Other objectives include assessing whether the early therapy with LPV-r, DRV-c FAV or HCQ can improve clinical composite scores (using a seven-category ordinal scale that has been already used in other COVID-19 trial) [37] and clinical biomarkers alteration associated with COVID-19.

5.3. Statistical hypothesis

The study is designed to assess two main hypotheses for defining antivirals superiority against a standard of care:

- **Virologic hypothesis.** Early treatment with antivirals increases the proportion of patients with undetectable SARS-CoV-2 in upper respiratory tract at day 7 after therapy form 25% to 50%;
- **Clinical hypothesis**. Early treatment with antivirals increases the proportion of participants who recover without need to hospital admission by the day 14 after therapy form 50% to 75%.

6. Study design

This study is a repurposing phase 3 adaptive trial implemented during a massive epidemic emergency.

In normal conditions, approval of new (experimental) drugs encompass different stages. Firstly, experimental drugs are tested in preclinical studies providing the primary evidence on potential efficacy *in vitro* and in animal models. Eventually, phase 1 and phase 2 study provide data on safety, pharmacokinetics and dosing. Finally, pivotal phase 3 trial are carried out to confirm drug efficacy and support registration for clinical use.

In the current circumstance of emergency, we looked for a strategy to shrink the process and to jump directly to phase 3. In particular, we used data produced by molecular docking analyses on PIs as a surrogate of preclinical stages, while repurposing makes it possible to make assumption on safety, pharmacokinetics and dosing. Thus, all PIs will be used as the same posology approved for anti-HIV therapy. Posology of FAV will be at the same used in previous clinical studies in China which reported good level of safety. [9-10] Posology of HCQ will be that suggested by Italian Drug Agency according to most recent evidence on patients with COVID-19. [38]

Adaptation is a carefully considered investigational procedure for modifying study parameters while the trial is ongoing, based on a review of the interim data analyses. [39-40] Our adaptive trial is a random controlled trial for parallel multiple comparisons. We aim to evaluate the safety and efficacy of four antivirals (LPV-r, DRV-c FAV or HCQ) in adult patients with mild-moderate COVID-19. The

study will be conducted in 5 Italian Provinces (Rome, Verona, Parma Piacenza and Cremona), but in principle, it can be immediately extended to other settings within and outside Italy.

The adaptive strategy includes:

A. parallel multi-arms (5 arms) open random comparison against a standard of care (no antivirals);
B. a 4-stage sequential design;
C. sample size re-calculation at each stage;
D. stopping rule for efficacy with a pick the winner strategy.

6.1. Parallel multi arm random allocation

Parallel multi arm random allocation is a widely used approach of multiple concurrent comparison of medical interventions. Parallel (i.e. concurrent) allocation between arms is particularly relevant in the circumstance of an ongoing epidemic due to a newly emergent virus whose potential mutations in the early phase of the epidemic might be associated to significant variation of the clinical presentation and degree of disease severity. We decided to conduct an open trial, as preparing placebo for the five treatment groups was not feasible in the short time.

6.2. Sequential design

Sequential design is a pivotal element of the adaptive strategy that allows for assessing participants data while the trial is ongoing for optimizing trial performance. In this study, we include a 4-stage procedure with three homogeneous interim analyses and one final analysis. Sequential design is always associated with inflation of statistical error due to multiple comparisons on the same accrued set of data. To control for this bias, we have defined *ad hoc* statistical plan and define adequate error spending function.

6.3. Sample size recalculation

The adequacy of sample size is important for clinical trials. However, in the current circumstance there are significant uncertainties about the sizes of parameters that are needed for optimizing study power. To prevent under-powering (i.e. leading to drop of efficacious intervention) and oversizing (leading to expanding time and cost of the study) we will implement a sequential recalculation of the sample size for each one of the three interim analyses.

6.4. Stopping rule (pick the winner)

We will adopt a pick the winner strategy. Pick the winner allows for selecting the best arm the soonest. According to this strategy, the trial will be terminated as soon as at least one active arm meets the criteria for superiority against the control arm. We will not include a stopping rule for futility because this strategy may inflate type II statistical error. Since toxicity of PIs, FAV and HCQ is predictably very low, we feel that potential inflation of statistical error is not counterbalanced by a significant advantage for participants.

7. Study population

Male and non-pregnant female adults aged 18 years or older with COVID-19 are eligible for the study. Children and adolescents will be excluded because COVID-19 clinical presentation in these patients is very mild. Thus, current knowledge about PIs, FAV and HCQ toxicity in children/adolescents does not counterbalance individual clinical advantage for COVID-19 treatment in these age classes. This selection could, however, be modified in an amendment, according to the evolution of knowledge on COVID-19 pathology and on the effectiveness of treatments against SARS-CoV-2 infection.

7.1. Case definition

For the purpose of the study the following definition are applied.

A **case of COVID-19** is a person with detectable both gene E and gene M from an adequate sampling of upper respiratory tract irrespective of clinical signs and symptoms.

A **mild-moderate case** is a case of COVID-19 who do not need immediate admission to hospital and has a national early warning score (NEWS) ≤ 2.

NEWS score has been revised on 2019 by England and Wales National Health Service as following [41]:

Table 1 NEWS scoring system

Element	Score						
	3	2	1	0	1	2	3
Respiratory rate	≤8		9-11	12-20		21-24	≥25
SpO_2	≤91	92-93	94-95	≥96			
Oxygen		Yes		No			
Systolic blood pressure	≤90	91-100	101-110	111-219			≥220
Pulse	≤40		41-50	51-90	91-110	111-130	≥131
ACVPU				A			C,V,P,U
Temperature, °C	≤35.0		35.1-36.0	36.1-38.0	38.1-39.0	≥39.1	

Score ≥3: discuss with duty nurse or senior colleague; score ≥6: immediate discussion with ACT advanced practitioner or ACT doctor. Concern about patient or difficulty obtaining any single parameter should lead to escalation regardless of score. Complete a sepsis screen on all patients with NEWS ≥3 with signs of infection.

ACT = acute clinical team; ACVPU = Alert, Confusion, Voice, Pain, Unresponsive; SPO_2 = peripheral capillary oxygen saturation; NEWS = National Early Warning Score.

8. Criteria for eligibility

8.1. Inclusion criteria

In order to be eligible for this study, a patient must meet all of the following criteria:

A. Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
B. Understands and agrees to comply with planned study procedures;
C. Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol
D. Male or female adult ≥18 years of age at time of enrolment;
E. Has laboratory-confirmed SARS-CoV-2 infection as determined by an approved molecular test (PCR) in Italy;
F. Being symptomatic for less than 5 days before starting therapy;
G. Do not meet criteria for immediate hospitalization (NEWS ≤ 2). [41]

8.2. Exclusion criteria

A. Requires immediate hospitalization or mechanical ventilation and/or supplemental oxygen therapy or have a NEWS ≥ 2;
B. Having already receive any of the trial drug less than 1 month before randomization
C. Being concurrently involved in another trial for COVID-19
D. Pregnancy (based on home test)
E. HIV infection (based on the anamnesis)
F. Use of any antiretroviral medication
G. Hypersensitivity to any of the used in the experimental compound including excipients
H. Use of medications that are contraindicated with chloroquine (Full list will be reported in the standard operative protocol to be developed in each of the five recruiting Centre).
I. Having a pacemaker and / or history or current evidence of clinically significant cardiac arrhythmia
J. Active or clinically significant cardiac disease including congestive heart failure (New York Heart Association Class III or higher)
K. Severe liver injury (Child-Pugh Class B or C);
L. Use of concomitant medications that prolong the QT/QTc interval (Full list will be reported in the standard operative protocol to be developed in each of the five recruiting Centre) [42]
M. Receiving drug that cannot be co-administered with any of the experimental compound (Full list will be reported in the standard operative protocol to be developed in each of the five recruiting Centre)
N. Autoimmune diseases receiving therapy at the time of randomization.
O. Women of childbearing potential and fertile men must agree to use at least one primary form of contraception for the duration of the study.

9. Intervention

Patient family doctor (general practitioner) supported by a dedicated infectious diseases specialist with experience in use of antiviral therapy will follow all patients at home.

9.1. Treatment arms

Control arm. No specific antiviral treatment.

ARM-1. DRV-c (Rezolsta) one film-coated tablet once a day with food for 14 days. Each film-coated tablet contains 800 mg of darunavir (as ethanolate) and 150 mg of cobicistat.

ARM-2. HCQ (Plaquenil) two coated tablets twice daily on day 1 and one coated tablet twice daily on day 2 to day 10. Each coated tablet contains 200 mg of hydroxychloroquine (as sulfate).

ARM-3. LPV-r (Kaletra) two film-coated tablet twice daily with food for 14 days. Each film-coated tablet contains 200 mg of lopinavir co-formulated with 50 mg of ritonavir.

ARM-4. FAV (Avigan) 9 coated tablets twice daily on day 1 and then 4 coated tablets twice on day 2 to day 10. Each coated table contains 200 mg of favipiravir.

9.2. Standard Patient monitoring

All patients will daily self-monitor their parameters and may call a doctor if needed. Patients will be provided with individual electronic device for measuring blood pressure, heart rate, body temperature and transdermal SpO2.

Home medical reassessment will be done if:

A. SpO2 ≤ 95%;
B. Systolic blood pressure >180mmHg or <100 mmHg
C. Body temperature >39°C or less than 35.0 °C;
D. Heart rate >110 bpm or less than 40 bpm;
E. Sensorial deterioration according to doctor's judgment.

Doctor may also decide to directly contact emergency service for immediate hospitalization for critical patients.

During the home visit doctor will regularly assess NEWS and evaluate the need for hospitalization. Patients will undergo a doctor visit regardless clinical conditions at the day of enrollment and eventually at day 7 at day 14 and at day 28. At each visit patients will be tested for viral shedding and will undergo blood test including hepatic function, renal function, coagulation, LDH, CPK and complete blood counts. Healthcare workers approaching COVID-19 patients will use personal protective equipment including a FFP3 (or FFP2) mask, gloves, gown and googles. FFP3 should be used always in case of any procedure on respiratory tract (including nasopharyngeal swab).

9.3. Other therapies allowed

Patients in the control group will receive no specific antiviral treatment. All other treatments including anti-hypertensive drugs, medications for diabetes (insulin and oral drugs), antibiotics, hormone therapy can be provided to patients according to medical judgments. Patients should not receive nonsteroidal anti-inflammatory drugs but can receive paracetamol if needed.

9.4. Safety monitoring and individual stopping rules

Any sign or symptom associated to drug adverse events will be daily reported daily during the telemedicine consult.

All patients will be censored when criteria for hospitalization are met. Another stopping rule includes drug related adverse events grade ≥ 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [43]

For all censored patients we will record the admission to the intensive care unit and the disease outcome (either survivor or non-survivors) by using the Regional surveillance systems.

Patients are free to withdraw from participation in the study at any time upon request, without any consequence. Patients should be listed as having withdrawn consent only when they no longer wish to participate in the study and no longer authorize the Investigators to make efforts to continue to obtain their outcome data. Every effort should be made to encourage patients to remain in the study for the duration of their planned outcome assessments. Patients should be educated on the continued scientific importance of their data, even if they discontinue the study. In the case of a patients becoming lost to follow-up, attempts to contact the patient should be made and documented in the patient's medical records.

10. Endpoints and Outcomes

10.1. Endpoints

Daily:

A. Systolic blood pressure (mmHg)
B. Diastolic blood pressure (mmHg)

C. SpO2 (%)
D. Body temperature (°C)
E. Heart rate (bpm)
F. Hospitalization in any day (NEWS ≥ 3)

At T0, T7; T14 and T28:

G. SARS-CoV-2 gene E (detectable or undetectable)
H. SARS-CoV-2 gene M (detectable or undetectable)
I. Complete blood counts
J. Hepatic function (ALT, AST, bilirubin tot and direct)
K. Renal function (BUN and creative)
L. Coagulation (PT, aPTT and INR)
M. Other marker including (D-dimer, CPK, LDH)
N. Seven-category ordinal scale

The seven-category ordinal scale has been already used in other COVID-19 trial [37] and consists of the following (qualitative) categories: 1 not hospitalized with resumption of normal activities; 2 not hospitalized, but unable to resume normal activities; 3 hospitalized, not requiring supplemental oxygen; 4 hospitalized, requiring supplemental oxygen; 5 hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 6 hospitalized, requiring ECMO, invasive mechanical ventilation, or both; 7 death.

10.2. Primary outcome

Virologic outcome. Proportion of participants with undetectable SARS-CoV-2 gene E and gene M at day 7 after randomization by arm.

Clinical outcome. Proportion of participants who need not hospitalization (NEWS ≤ 2) by day 14 after randomization by arm.

10.3. Other outcomes

Proportion of participants with undetectable SARS-CoV-2 gene E and gene M at day 14 after randomization, by arm.

Proportion of participants with undetectable SARS-CoV-2 gene E and gene M at day 28 after randomization, by arm.

Proportion of participants who need not hospitalization (NEWS ≤ 2) by day 7 and 28 after randomization, by arm.

Proportion of patients in each category at point N at time 0, 7, 14 and 28, by arm

Mean value of category at point N by arm at time 0,7,14 and 28

Proportion of participants with any adverse event (grade ≤ 2 according to CTCAE) at day 7, 14 and 28, by arm.

Proportion of participants with severe adverse events (grade ≥ 3 according to CTCAE) at day 7, 14 and 28, by arm.

Mean variation of value of biomarker parameters reported in end point section (point I to M) from base line to day 7, 14 and 28 after randomization, by arm.

Proportion of participants admitted to intensive care at day 7, 14 and 28 after randomization, by arm.

Proportion of survivors at day 7, 14 and 28 after randomization by arm.

11. Methods

Randomization.

Patients will be individually randomized at central level. All recruiting center will have access to an enrollment and monitoring on-line application crated by using the Research Electronic Data Capture software by Vanderbilt University (REDCap). REDCap is a browser-based, metadata-driven EDC software and workflow methodology for designing clinical and translational research databases. It is widely used in the academic research community: the REDCap Consortium is a collaborative, international network of more than 2400 institutional partners in over 115 countries, with more than 590,000 total end-users employing the software for more than 450,000 ongoing research studies. The University of Verona will develop the forms for patients' enrollment and follow-up. The randomization sequence will be generated by STATA 15 software.

11.1. Blinding

Not applicable

11.2. Electronic case report form

Patients' data will be recorded in an ad hoc online database developed REDCap. The Electronic case report form will be developed by University of Verona and shared online among all participating centers. The enrollment will start as soon as the electronic case report form is approved by all participating centers.

11.3. Steering committee and adaptive binding decision

Steering committee will include one representative from all units participating to the study and two independent international experts. The Steering committee will be chaired by the INMI's Scientific Director. The Steering committee will oversee all the aspects of the project's life: decision about safety, adaptive change binding decision for stopping rule/sample size recalculation, diagnostics issues, capacity development, financial, schedule, partnership, dissemination and exploitation. The Steering committee will hold at least one meeting a week on teleconference. In addition, extraordinary sessions will be held in case of critical issues. INMI's Scientific Director who chairs the Steering committee is in charge to call extraordinary session of the Steering committee.

12. Statistical plan

This is a multi-arm parallel randomized controlled clinical trial. We set two experimental hypotheses:

- **Virologic hypothesis.** Early treatment with antivirals increases the proportion of patients with undetectable SARS-CoV-2 in upper respiratory tract at day 7 after therapy form 25% to 50%;
- **Clinical hypothesis**. Early treatment with antivirals increases the proportion of participants who recover without need to hospital admission by the day 14 after therapy form 50% to 75%.

If either one of these experimental hypotheses is true, the study has a two tails alpha-error of <0.05 and an overall power of >80%.

12.1. Sequential design procedures

The sequential design includes 5-arm 4-stage procedure allowing for (binding) early stopping rule according to efficacy of at least one active arm (pick the winner strategy). Alpha spending function is according to normal approximation test combination with constant significance level and constant information rate across the stages. We do not make assumption on variance in each arm. Bonferroni method is used to adjust for multiple comparisons.

The probability to find an effective therapy according to the variability of observed efficacy of the best arm has been calculated by simulation (10,000 iterations).

Figure 1 and Figure 2 report average sample size, the overall probability to find an effective treatment and early stopping for experimental virologic hypothesis.

Figure 3 and 4 report average sample size, the overall probability to find an effective treatment and early stopping for experimental clinical hypothesis.

12.2. Sample size recalculation

Minimum and maximum sample size will significantly change according to the observed effect within the trial sample (expected **average** sample size is between 175 and 435 participants). In particular, we expect to enroll 35 participants per arm at the first stage and then between 16 and 70 for each eventual stage if the stopping rule is not met. The actual number of new participants in each arm will be calculated according to the observed efficacy of the best arm at each interim analysis with an overall conditional power of 90%. This approach allows either to minimize the number of enrolled participants if the experimental hypothesis is too conservative or to have a good power level if the experimental hypothesis is too optimistic. The overall average sample size according to virologic hypothesis and clinical hypothesis are reported in figure 1 and figure 3, respectively.

12.3. Analysis of efficacy (primary outcome)

Interim analysis and final analysis will be carried out by taking into account the potential effect of each individual component of the adaptive design. The interim analysis will provide:

A. estimate of efficacy as risk difference and relative 95% CI
B. criteria for stopping rule
C. if the stopping rule is not met the analysis will provide the number of participants to be randomized for the subsequent stage in each arm.
D. final analysis will provide efficacy as difference estimate of efficacy as risk difference and relative 95% CI

12.4. Analysis of secondary outcome

Binary variables including need for hospitalization according to NEWS score and virologic status at T14/T28 will be modelled according to separate logistic regression models to assess the potential effect of each different treatment arm. All models will be adjusted for the effect of age and gender.

The analysis of repeated quantitative variables at T0, T7 , T14 and T28 will be modelled according to mixed regression model with standard intercept at patients level and random slope at time level. This includes blood counts parameters (WBC, Lymphocytes, Neutrophils, Eosinophils, Platelets and hemoglobin), hepatic function parameters (ALT, AST and bilirubin), renal function parameters (BUN and creative), coagulation parameters (PT, aPTT and INR) and other markers (D-dimer, CPK, LDH). For all of these variables a kinetic curve will be produced adjusting for age, gender, arm allocation and virologic status of the patient.

12.5. Software for simulation and analysis

Study design, simulations, interim analysis and final analysis of primary outcome will be carried out by ICON ADDPLAN V 6.1. This is a proprietary statistical package that contains approved algorithm for dealing with the adaptive design according to EMA and FDA standards. The analysis of secondary outcomes will be carried out at the end of the trial and will be carried out by STATA V.15.

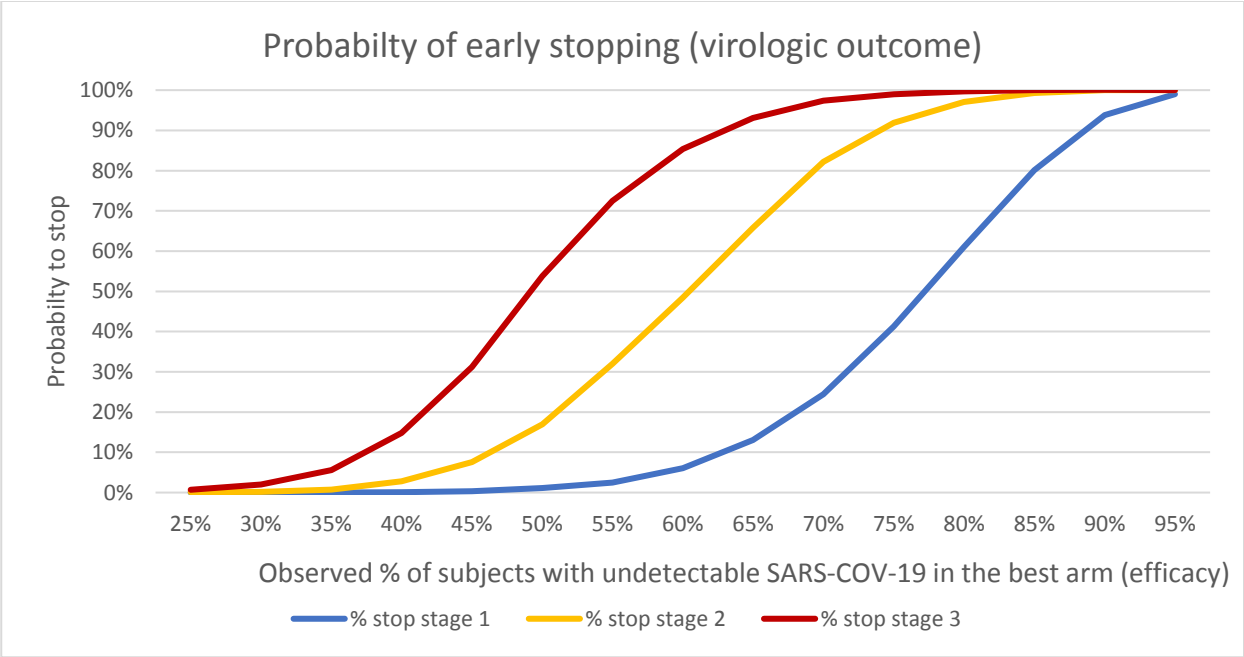

Figure 1 Cumulative probability of early termination of the trial according to the virologic outcome. Estimates are calculated assuming that observed proportion of participants who have undetectable SARS-COV-19 in upper respiratory tract at day 7 after randomization in control arm is 25%.

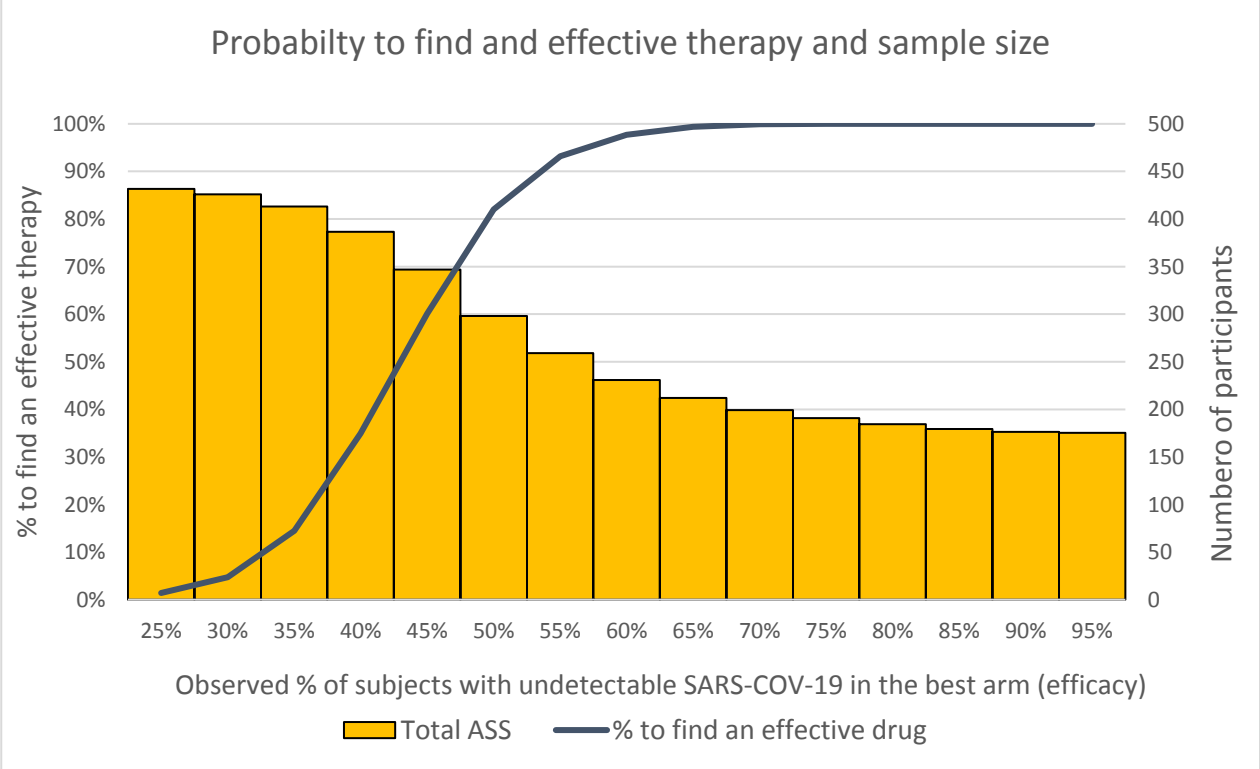

Figure 2 Probability to find an effective intervention and relative average sample size (ASS). Estimates are calculated assuming that observed proportion of participants with detectable SARS-CoV-19 in control arm is 25%.

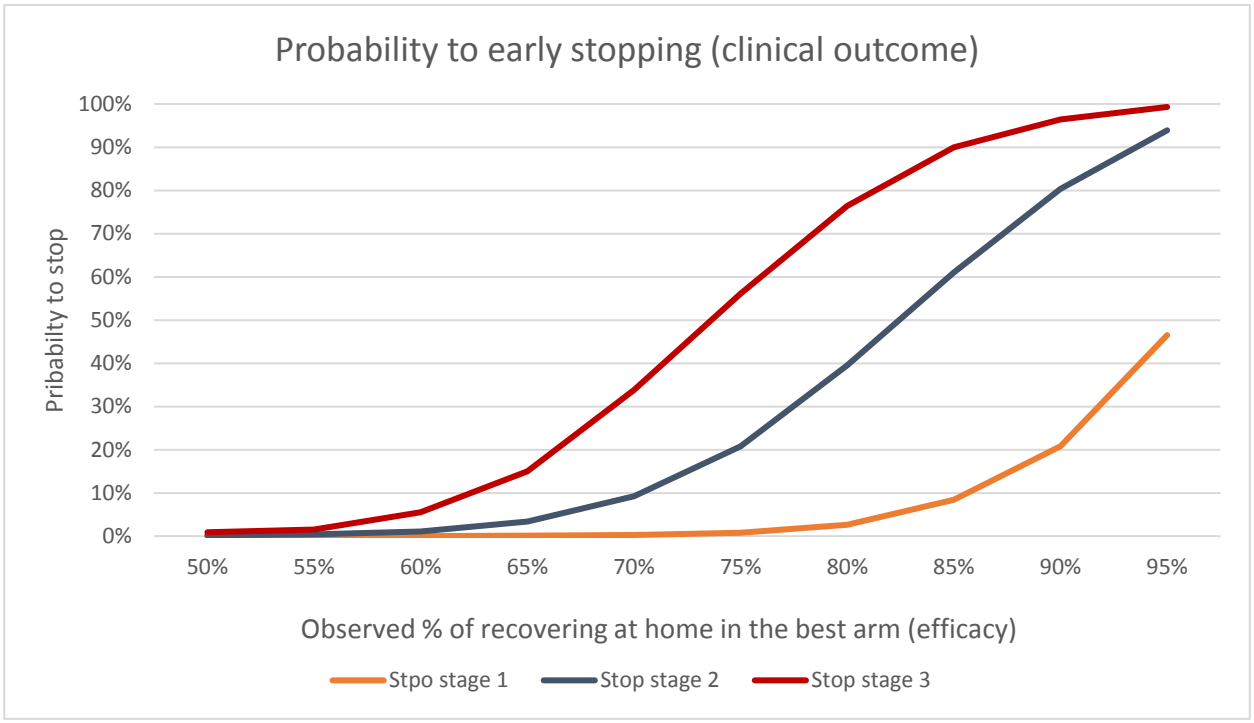

Figure 3 Cumulative probability of early termination of the trial according to clinical outcome. Estimates are calculated assuming that observed proportion of participants who do not need hospitalization by day 14 after randomization is 50%.

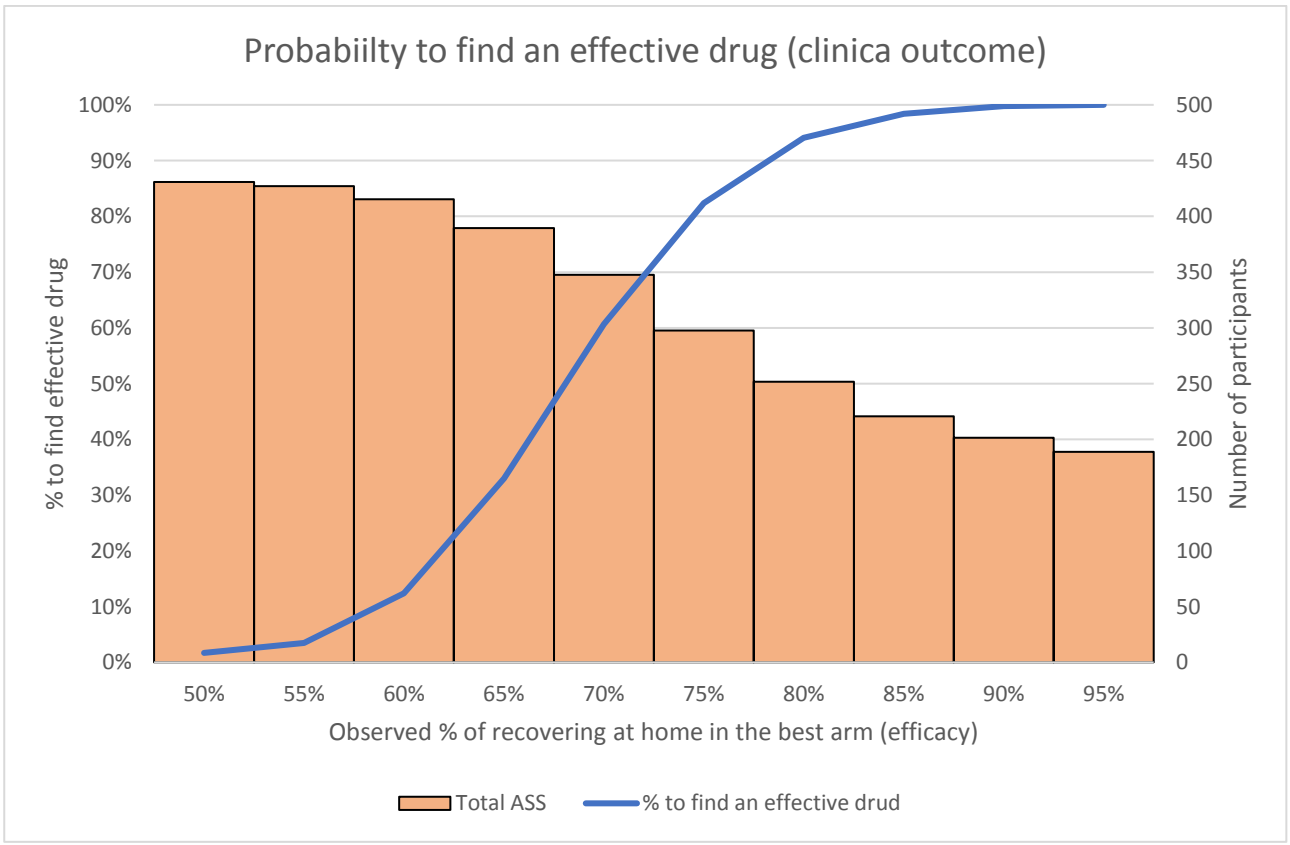

Figure 4 Probability to find an effective intervention and relative average sample size (ASS) according to clinical outcome. Estimates are calculated assuming that observed proportion of participants who do not need hospitalization by day 14 after randomization is 50%.

13. Timing

The estimated time to set up local infrastructure for the trial is about 2 weeks. This includes:

A. implementation of database for the electronic case report form
B. development of protocol and standard operative procedure
C. approval by ethical board of all protocols
D. institution of and external steering committee

We expect to enroll about 10 patients per day. If the virologic hypothesis is true, we need an average sample size of about 300 participants, the enrollment will be complete within about 4-5 weeks and the follow-up of the last patient will be concluded by week 9-10. The final analysis will be carried out the latest with 1-2 weeks after trial termination. The expected time to carry out the entire study is about 11-12 weeks. The application of early stopping rule may significantly reduce the overall enrollment time (see statistical plan and simulation).

Table 1. Gantt chart

Activity	Time in weeks											
	1	2	3	4	5	6	7	8	9	10	11	12
Protocols	■											
Database ECRF		■	■									
Approval		■										
Steering committee	■	■	■	■	■	■	■	■	■	■	■	■
Interim analysis					■		■		■			
Enrollment				■	■	■	■	■				
Follow-up				■	■	■	■	■	■	■	■	
Final analysis												■

14. Feasibility

At present about 100 new confirmed cases of COVID-19 are reported each day in Rome, of them between 40% and 50% do not need hospitalization and are eligible for the study. Similar data are reported in other Italian province participating to the study.

The project is based on the institution of a network of experts, clinical units, the support of Regional health services of and the association of general practitioners (*Medici di Mecidina Generale e Pediatri di Libera Scelta*). All the participants are currently directly involved in the management of COVID-19 outbreak in Italy and strongly motivated to participate. INMI Lazzaro Spallanzani (coordinating center) has a longstanding experience in multicenter studies and its Ethical Committee serves as the National Central Committee for approval of COVID-19 clinical study to be carried ou in Italy.

According to the current systems of surveillance implemented in different Region in Italy, all the COVID-19 cases who do not need immediate hospital admission are monitored at home. All participants will be provided with pulse-oximetry device, automatic arm blood pressure monitor and device for internet access. All participating units will provide access to participants to a direct consultation with a GP. Besides GPs who are in charge for caring and for enrolling patients will have direct access to direct consultation with an infectious diseases specialist.

Experts involved in the project have different background and they have a strong experience in the fields of infectious diseases and family medicine. The adaptive randomized study will be embedded

into the Regional surveillance system of COVID-19 of the participating units. Investigators are expected to enroll patients and provide them with identical high standard of care and equal opportunity to participate to the study.

Clinical coordinator form each recruiting centers are expected to discuss with their Regional authority any modification to legislative framework needed for implementing the study.

15. Good clinical practices and ethics

15.1. Good clinical practice

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki, in the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and in the appropriate regulatory requirements. The drugs to be used in this trial are already registered and all investigators are already familiar with these drugs since they are largely used in HIV treatment. In order to minimize the risk of adverse events the following strategies will be used:

A. **Patient information**. All patients will be provided with oral and written information on the possible adverse events associated with the use of study drugs.
B. **Review of eligibility**. Prior to randomization participating unit will fill a checklist in order to allow verification of eligibility criteria.
C. **Monitoring**. Laboratory data and clinical symptoms will be checked during treatment. A total of four lab monitoring visits and daily teleconsultation will be performed for each participant.

15.2. Ethical aspects

The Independent Ethics Committee (IEC) of the institution of the Principal investigators will serve a coordinating IEC according to the Italian law. Approval must be granted by the IECs of the coordination Unit only according to the current emergency. The Steering Committee will provide information to IEC 14 days.

15.3. Insurance

The study will be conducted according to the law about the study insurance agreement (DM 14 luglio 2009); ad-hoc insurance will be established.

16. Budget *(in Italian)*

Il budget è stato sviluppato tenendo conto della forma adattiva del disegno di studio. Tutte le valorizzazioni economiche sono state fatte per la *best guess* del disegno che prevede l'arruolamento ed il follow-up per 4 settimane circa 300 soggetti, ovvero circa 1,300 euro per paziente. Il disegno adattivo tuttavia prevede per sua natura oscillazioni significative (tra 175 e 500 pazienti *on avarage*) e va pertanto considerato che la reale spesa può variare effettivamente tra circa 420.000 a circa 1.200.000 euro.

Materiale / Utilità	costo unitario	numero per paziente	quantità	totale	Descrizione
Costi coordinamento medico	75000.00	na	**2**	150,000.00	**2 dirigenti medici per un anno**
pulsiosimetro	25.00	1	300	7,500.00	Strumentazione da offrire individualmente a domicilio
sfingomanometro elett.	20.00	1	300	6,000.00	Strumentazione da offrire individualmente a domicilio
DPI completo con FFP3	9.00	5	1500	13,500.00	5 visite (4 standard + 1 extra)
Assisntenza domicilaiare	500.00	1	300	150,000.00	100 euro a settima per paziente
Idrossiclorochina (Plaquenil)	0.20	22	60	264.00	Prezzo ex fatctory IVA esclusa
Lopinavir (Kaletra)	2.50	56	60	8,400.00	Prezzo ex fatctory IVA esclusa 1 confezione 2 trattamenti
Darunavir (Rezolsta)	30.00	14	60	25,200.00	Prezzo al pubblico per terapia di 14 compresse
Favipiravir (Avigan)	1.00	na	15000	15,000.00	Concesso in stock da FUJIFILM per circa 150 trattamenti (15.000 compresse)
Emocromo	4.00	4	1200	4,800.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
ALT	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
AST	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
Bilirubina tot e Fraz.	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
Creatinina	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
Azotemia	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
CPK	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
Coagulazione	11.00	4	1200	13,200.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
D-Dimero	6.00	4	1200	7,200.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
LDH	2.00	4	1200	2,400.00	Costo indicativo tra regioni per 4 misure (T0, T7, T14 e T28)
test di gravidanza	7.00	1	150	1,050.00	
test per G6PD	7.00	1	300	2,100.00	
Sars-CoV-2 PCR	90.00	3	900	81,000.00	Costo intdicativo tra regioni per misure a T7 , T14 e T28 (le misure a T0 sono pre randomizzazione)
Sviluppo piattaforma	na	na	na	0	REDCAP concesso da Vanderbilt University / Università di Verona
Assicurazione	na	na	na	51,590.00	
Costi generali	na	na	na	100,000.00	Costi amministrativi per la struttura coordinatrice
Costi generali	na	na	na	68,000.00	Costi generali per il mantenimento dei Board di esperti
Totale			0	721,604.00	Costo per 350 arruolamenti e relativo follow-up

17. Institution agreement

The Principal Investigator will submit in the Clinical Trials all the documentation required by law to AIFA, as the Competent Authority and to Ethics Committee within a week after approval.

Also, the principal investigator will comply in all respects with:

A. the contents of this clinical study protocol
B. the standards of Good Clinical Practice as defined in the "Note of Guidance on Good Clinical Practice (CPMP/ICH 135/95)" and related Guidelines
C. all applicable regulatory requirements including national drug law and data protection law.

18. Principal investigator and key persons

Simone Lanini. He will coordinate study design, study implementation and data analysis.

Evelina Tacconelli. She will supervise the activity in the study unit of Verona and will develop randomization and CFR systems.

Giovanna Scroccaro. She will supervise the activity in the study unit of Verona.

Roberta Ioppi. She will supervise the activity in the study unit of Verona.

Chiara Montaldo. She will supervise patient's enrollment and data collection in Lazio and support communication between the units.

Andrea Antinori. He will supervise clinical aspect.

Enrico Girardi. He will supervise ethical and methodology aspect.

Carlo Ferrari. He will supervise the activity in the study unit of Parma

Stefano del Canale. He will supervise the activity in the study unit of Parma

Angelo Pan. He will supervise the activity in the study unit of Cremona

Luigi Cavanna He will supervise the activity in the study unit of Piacenza.

Aurora Di Filippo as a member of Scientific Committee (AIFA)

Maria Paola Trotta as a member of Scientific Committee (AIFA)

Francesco Trotta as a member of Scientific Committee (AIFA)

Giuseppe Ippolito. He will supervise the study and chair the steering committee.

19. Participating centers

INMI Lazzaro Spallanzani Roma Italy

Azienda Ospedaliera-Universitaria Integrata di Verona, ULZ 9

Azienda USL and Azienda Ospedaliero-Universitaria di Parma

Azienda USL, Ospedale di Piacenza

ASST Cremona

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